Pneumococcal meningitis: heterogenous receptor expression and the influence on *Streptococcus pneumoniae* interaction with the blood-brain barrier

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1. Introduction

Streptococcus pneumoniae (the pneumococcus) is a Gram-positive human pathogen that can cause life-threatening invasive diseases such as pneumonia, bacteremia and meningitis. How *S. pneumoniae* traverses the endothelial cell layer of the blood brain barrier is currently unclear. In this study, we address the question how *S. pneumoniae* crosses the blood brain barrier.

2. Methods

Mice were intravenously infected with *S. pneumoniae*, and were sacrificed at various time points after infection to mimic the stages preceding meningitis. Bacterial localization and endothelial cells were detected on brain tissue cryostat-cut slides using immunofluorescence. Subsequently the location of various host receptors known to play a role in the interaction with *S. pneumoniae* was determined using immunofluorescence.

3. Results

Co-localization of *S. pneumoniae* within the vessels of the blood-brain barrier occurred at specific anatomical sites within the brain over time. Confocal analysis confirmed that *S. pneumoniae* is tightly associated with the endothelial cells. Analysis of the systemic and local immune response indicated marked differences in the local response of the brain. Analysis of the location of various known host cell receptors indicated a heterogenous expression of these receptors on endothelial cells in the brain. Currently, the consequences of this heterogeneity for the interaction with the bacteria are further investigated using IF and *in vitro* assays.

4. Conclusions

S. pneumoniae is attached to both the macro- and microvascular endothelium in the mouse brain depending on the anatomical site. The presence of bacteria in the blood elicits markedly different responses in the brain compared to the systemic response. Furthermore, there was a marked heterogeneity in the expression of known host cell receptors for *S. pneumoniae* in the brain. Ultimately, this work will lead to a better understanding of how pneumococci interact with the blood brain barrier and cause meningitis.

Biofilm Formation Avoids Complement Immunity and Phagocytosis of *Streptococcus pneumoniae*

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ABSTRACT:

Colonization of the nasopharyngeal tract is a first and necessary step in the infectious process and often involves the formation of sessile microbial communities by the human pathogen *S. pneumoniae*. The ability to grow and persist as biofilms is an advantage for many microorganisms because biofilm-grown bacteria show a reduced susceptibility to antimicrobial agents and hinder the recognition by the immune system. Using pneumococcal strains growing as planktonic cultures or as biofilms, we have investigated the recognition of *S. pneumoniae* by the complement system and its interactions with human neutrophils. Deposition of C3b, the key complement component, was impaired on *S. pneumoniae* biofilms. In addition, binding of CRP and the complement component C1q to the pneumococcal surface was reduced in biofilm-growing bacteria demonstrating that pneumococcal biofilms avoid the activation of the classical complement pathway. Besides, recruitment of factor H, the down-regulator of the alternative pathway by a PspC-mediated mechanism was enhanced by *S. pneumoniae* growing as biofilms. Furthermore, phagocytosis of pneumococcal biofilms was also impaired. The present study confirms that biofilm formation in *S. pneumoniae* is an efficient way for host immune evasion both from the classical and the PspC-dependent alternative complement pathways.

Keywords: biofilm, phagocytosis, complement system.

1. Introduction

The growth and dispersal of microbes, whether pathogenic or environmental, commonly involve the production of biofilms, which represent the primary mode of pneumococcal growth during colonization, recurrent otitis media and the early stages of invasive disease [1]. These evidences support the importance of studying pneumococcal sessile communities to understand key events in the pathogenesis development of this important human pathogen.

Biofilm formation is a complex process initiated by the attachment of microorganisms to a surface or interface that is embedded in an extracellular matrix constituted by various polymeric substances [2]. The biological and physicochemical characteristics of biofilm structure protect the bacterium from environmental adversities and confer the microorganism an inherent resistance to antimicrobial therapies and the host immune response [3]. The complement system represents one of the first lines of defense against invading pathogens such as *S. pneumoniae* and plays a vital role in both innate and acquired immunity [4]. This unique host defense mechanism is activated by three different pathways —known as the classical, alternative and lectin pathways— that converge at the central com-ponent C3. This component is involved in es-sential phases of the immune response such as recognition and clearance of microorgan-isms, inflammatory response and induction of phagocytosis.

2. Results

2.1. C3b deposition on *S. pneumoniae* growing as biofilms or planktonic cultures

The deposition of the complement component C3b on the surface of *S. pneumoniae* was investigated by a flow cytometry assay using bacteria grown either as biofilms or as planktonic cultures. C3b deposition on pneumococcal biofilms was markedly impaired in comparison to planktonic cultures.

2.2. Reduced activation of the clas-sical complement pathway by pneumococcal biofilms

The classical complement pathway is activated by the recognition of antigen-antibody complexes on the bacterial surface by the component C1q and it is generally considered to be an effector of the acquired immune response. In addition, recognition of *S. pneumoniae* by acute phase proteins such as CRP, increases the deposition of C1q on the pneumococcal surface activating therefore the classical pathway. Binding to pneumococcus by CRP and C1q was reduced when the bacteria were grown as a biofilm demonstrating that biofilms enhance the resistance of *S. pneumoniae* to complement immunity by diminishing the classical pathway activation.

2.3. Recruitment of human complement regulators

Interaction with the major fluid-phase regulators of either the classical or alternative complement cascades was investigated by flow cytometry. Deposition of C4BP, the main down-regulator of the classical pathway is not affected by biofilm formation. In contrast, recruitment of the downregulator of the alternative pathway, factor H, by a PspC mediated mechanism was enhanced by *S. pneumoniae* growing as biofilms.

2.4. Phagocytosis by neutrophils is impaired in *S. pneumoniae* biofilms

The susceptibility of pneumococcal biofilms to the opsonophagocytosis process mediated by human neutrophils was markedly impaired in comparison to the planktonic culture, demonstrating that the sessile growth of *S. pneumoniae* represents an advantage to avoid the recognition and phagocytosis proc-ess mediated by human neutrophils.

3. Conclusion

Overall, our study indicates that biofilm formation may constitute an advantage in certain phases of the pneumococcal pathogenic process such as nasopharyngeal colonization or during the early steps of microbial attachment for invasion by avoiding the host immune system to efficiently recognize and destroy *S. pneumoniae*.

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Phosphoglycerate kinase of *Streptococcus pneumoniae* binds angiostatin and tissue-type plasminogen activator

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ABSTRACT:

Streptococcus pneumoniae binds the fibrinolysis proenzyme plasminogen (PLG). Here, we identified the phosphoglycerate kinase (PGK) as PLG binding protein specifically interacting with angiostatin, representing the N-terminal kringle domains 1-3. Electron microscopic analysis confirmed surface expression of PGK irrespective of capsule expression. PGK binds human and murine PLG with high affinity as shown by Surface Plasmon Resonance (SPR) technique. Results of peptide array analyses detected two PLG binding sites (BS1 and BS2). In addition to PLG, PGK also directly binds the tissue-type plasminogen activator (tPA). These results point to the high relevance of plasminogen binding for *S. pneumoniae* – host interaction.

Keywords: angiostatin, phosphoglycerate kinase, plasminogen, tissue type plasminogen activator.

1. Introduction

Pneumococci bind PLG via a subset of surface exposed proteins including the glycolytic enzyme enolase [1]. PLG is the proenzyme of the major fibrinolysis enzyme plasmin. The host-derived PLG activators tPA and urokinase-type PLG activator (uPA) convert PLG on the surface of pneumococci into the protease plasmin. This surface-exposed proteolytic activity facilitates pneumococcal transmigration through fibrin thrombi and extracellular matrices [2]. The 92 kDa PLG is composed of five homologous kringle domains followed by an enzymatic domain. K1-3 and K1-4, respectively, comprise angiostatin.

2. Methods and Results

2.1. Surface-detection of PGK

Recombinant PGK was generated in *E. coli* to subsequently produce antigen-specifc antibodies. Following immune detection using electron microscopic visualization detected PGK on the surface of encapsulated and non-encapsulated isolates (Fig.1).